THE

BIOLOGICAL EXAMINATION

OF

WATER.

BY

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BIOLOGICAL EXAMINATION OF WATER.

In the Reichs Gesundheits Amt, Berlin,—where, through the courtesy of Prof. Koch, the late Director, I have been permitted to study for some months,—a large number of water analyses are made, special importance being attached to the bacteriological examination. The late Dr. Angus Smith, of Manchester, has described in the *Sanitary Record* a method of examining water for the presence of micro-organisms, based upon Dr. Koch's earlier researches, but the system now in force in the Laboratories of the German Imperial Board of Health differs materially. It is not a mere qualitative examination for the detection of micro-organisms, but one by which an approximate determination of the number present is attained, and it may therefore perhaps be of interest if I describe at length the *technique*.

In England, unfortunately, at the present time there is no institution where Medical Officers of Health and others can receive instruction similar to that afforded at the Gesundheits Amt, Berlin; and in this communication I assume that the reader has had no previous experience with recent methods of bacteriological research. I have, therefore, thought it advisable to enter rather fully into minutiæ which otherwise might have been omitted. Success in bacteriological work is largely dependent upon close attention to details: the methods are simple, but their correct performance is often from this cause difficult of attainment. The *rôle* which micro-organisms play in the causation of disease is year by year becoming better defined; and the water analyst who wishes to be abreast of the times cannot now confine his attention solely to the "organic" and "mineral" constituents of a water, his attention must also be directed to the *organised*; and not so much to the larger organisms, visible to the naked eye, but to the smaller forms, the Bacterium and other allied species. The importance of examining a potable water for micro-organisms cannot be over-estimated. There is abundant evidence to justify the belief that the germs of at least two diseases—cholera and typhoid fever—are fre-

quently conveyed by water. The addition of one drop of a cholera stool to a litre of sterilised water would form a mixture which, if examined by the Wanklyn, Frankland, or any other chemical method, would yield absolutely negative results. The analysis of a stronger mixture would show the presence of so much "free" and "albumenoid ammonia," or "organic" carbon and "nitrogen," but beyond that no information would accrue. The bacteriological examination would, on the other hand, in either case, with absolute certainty demonstrate the presence of a comma-shaped micro-organism, while subsequent cultivation experiments would indicate whether the organism was the cholera bacillus or not. I do not wish it to be understood that I consider the chemical examination of a potable water useless: the bacteriological test is not by any means intended to supersede chemical methods; it is only an additional test, but one supplying information which a chemical analysis does not afford.

Briefly, Prof. Koch's method consists in adding a measured volume of water to sterilised liquid meat peptone gelatin, which is then poured on a glass plate, and after a certain period the developed colonies of micro-organisms counted, examined microscopically, and, if necessary, cultivated in various media and under different physical conditions. In describing the method I propose first giving a list of the apparatus required; (2) method of preparing the reagents; (3) precautions to be adopted in the collection of samples of water; (4) analytical process; (5) inferences to be drawn from the results.

I. *Description of Apparatus.*

The apparatus used for the bacteriological examination of water are simple, and with a few exceptions are to be found in every chemical laboratory. The only expensive instrument is the microscope; the remainder are all inexpensive.

Test-tubes.—The most suitable size is 160 × 15 m.m. The tubes are employed for containing the gelatin solution, and are prepared for use in the following manner:—They are rinsed with a small amount of strong hydrochloric acid, and thoroughly washed with water, care being taken to remove all trace of acid. The tubes are inverted, and allowed to drain and dry. When quite dry they are plugged moderately tight with cotton-wool. The plug should extend about three-quarters of an inch within, and the same distance beyond the mouth. The tubes are then packed in a *wire basket* (Fig. 1), with the plugged mouths upwards, and placed in a *hot-air bath* (Fig. 2),

Fig. 1.

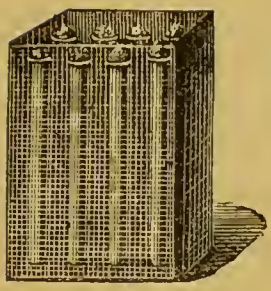


Fig. 2.

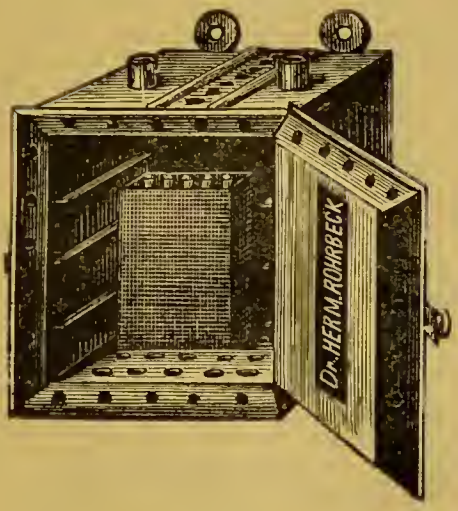


Fig. 3.

which should be heated to 150° to 160° C. for one hour and a-half. This temperature slightly browns the plugs, and is sufficiently high to destroy any germs which may have adhered to the interior of the tubes or to the plugs. After the lapse of the prescribed time the burner should be extinguished, and the tubes allowed to cool in the oven with the door closed: when cold they can be removed from the cage and preserved in a box, care being taken to avoid disturbing the plugs.

Pipettes to hold 1, 2, and 3 c.c., and also to hold 1 c.c., divided into one-tenth and one-fifth of a c.c., are used to measure the water. They are sterilised by being heated in the air-bath to 150°—160° C. for one hour and a half, and are then allowed to cool in it, and removed for use as required. Several pipettes should be provided, as a freshly sterilised one is requisite for each sample of water tested. It must be remembered that a pipette once sterilised will not remain germ-free for any time, though not removed from the bath in which it has been heated. The most convenient procedure is to daily heat the requisite number of pipettes required.

Glass rods of about 2 c.m. in length are used for spreading the liquefied gelatin on the plates, &c. They are sterilised in the same manner and at the same time as the pipettes.

Glass plates of flat crown-glass free from flaws, 13 × 10 c.m. in size, are used for receiving the gelatin film. The sharp edges should be ground down, and the plates then carefully freed from grease, rinsed with hydrochloric acid, and thoroughly washed. When dry they are placed in a copper or sheet-iron box (Fig. 3) provided with a tightly-fitting cover, which is closed, and the box heated in the air-bath for two hours to 150°—160° C. When cold the box is removed from the bath, and provided care be taken in the removal of a plate the glasses will remain fit for use for several days.

In removing a glass plate the box should be held horizontally, a plate slipped out, and the cover at once replaced. If a plate has not been recently sterilised it is advisable to again heat it, by holding one corner with a pair of crucible tongs, and rapidly passing both sides several times through a flame, so as to thoroughly heat every part. It is then placed under a bell-jar to cool, supported on a beaker or other convenient support. A warm plate must never be placed on the levelling apparatus (Fig. 7) or it is almost certain to crack.

Dr. H. Rohrbeck, of 100, Friedrich-strasse, Berlin, manufactures glass plates, with a slightly raised square ridge in the centre, 9×9 c.m., into which the gelatin,

FIG. 4.

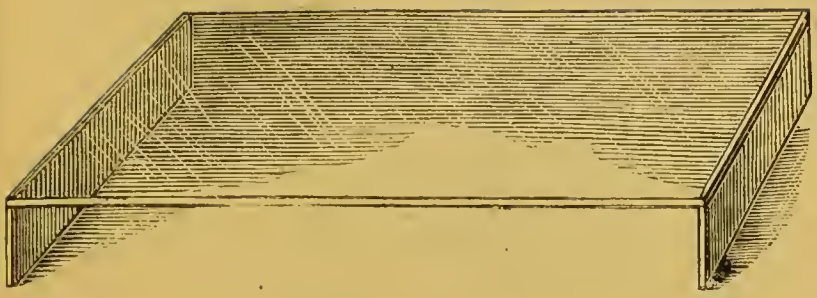


FIG. 5.

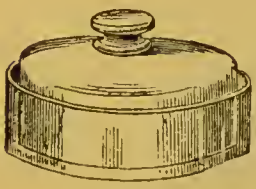


FIG. 6.

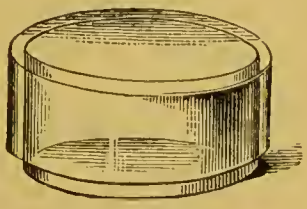
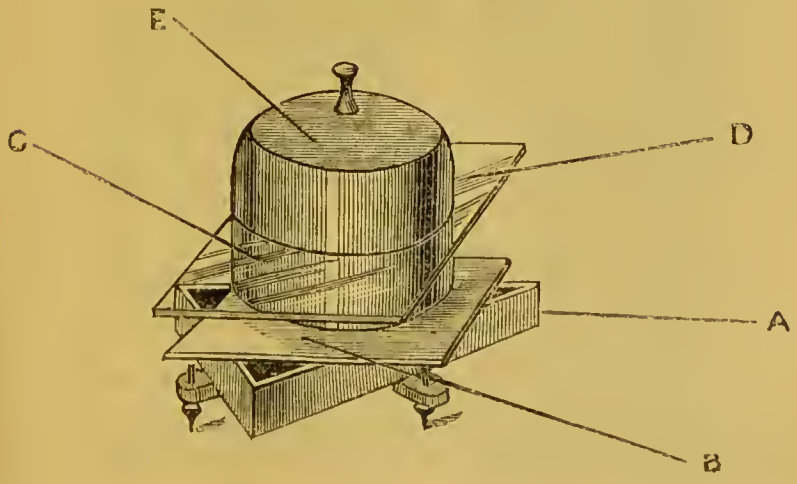


FIG. 7.



after admixture with the water, is poured. These plates can be sterilised either in the hot-air bath or by direct application of a flame, without the ridge cracking. They are useful for water analysis, as the gelatin can be accurately spread over the required surface.

A box similar in construction to Fig. 3 may be employed for holding the pipettes and glass rods during sterilisation.

Glass benches (Fig. 4), consisting of a piece of sheet-glass, 13 × 10 c.m., with two slips of thick plate-glass fixed at the ends by means of sealing-wax, are used for supporting the glass plates after they have been coated with gelatin. Before being used the benches are washed with a solution of aqueous corrosive sublimate, 1 to 1000, and allowed to drain.

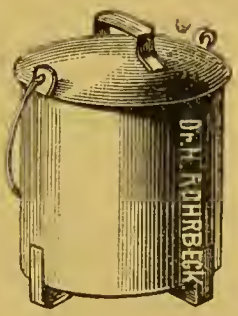
The coated gelatin plates, supported on the glass benches, are placed in a *moist chamber* for development of the bacterial colonies. In the Gesundheits Amt, glass vessels (Figs. 5 and 6) are used. They are prepared for use by being well rinsed with sublimate solution. A piece of thick circular blotting-paper is then fixed in the cover, and two or more layers on the bottom of the receiver, which are well wetted with sublimate solution, and the superfluous liquid allowed to drain off. The object of the wet blotting-paper is to keep the air in the interior of the vessel saturated with moisture, and the paper on the cover also prevents any drops of condensed moisture from falling on the plates. Should the cover fit into and not rest on the surface of the receiver, as in Fig. 5, care should be taken that the blotting-paper at the bottom does not project beyond the edge of the cover, else that portion of the paper exposed to air would dry rapidly, and by capillary action remove moisture from the central parts of the paper. These glass chambers are capable of holding five to six glass plates with benches. Glass chambers, though convenient, are somewhat expensive, but fortunately admit of being replaced by ordinary soup-plates. The soup-plates for this purpose should, if possible, be of two sizes, the one used as the receiver being deeper and larger than the one used as the cover. The soup-plates are prepared for use in precisely the same manner as the glass receivers and covers, and they are capable of holding three plates and benches.

In pouring the liquefied gelatin on a plate after admixture with the sample of water, it is requisite that the plate should be supported on a level surface; and, secondly, that there should be some contrivance by which the gelatin film may be exposed to a low temperature to accelerate the setting. These desiderata are attained by use of the following apparatus (Fig. 7). A consists of a tri-

angle of either metal or wood, provided with three levelling screws. The triangle supports a thick glass plate, B, on which rests a circular glass vessel, C, with the rim ground perfectly flat. The glass receiver is about one-half filled with small lumps of ice, and water added to overflowing. A sheet of thick plate glass, D, is then pushed over the surface in such a manner that the iced water is in actual contact with the under surface of the plate. A small circular spirit-level is now placed on the centre of the plate, and the apparatus levelled by means of the adjusting screws. In using the apparatus, the glass plate which is to receive the liquefied gelatin is placed on the plate D, and after the gelatin has been poured on it, it is covered with the bell-jar, E, until the gelatin has solidified.

FIG. 8.

FIG 9.



The apparatus may be advantageously modified by substituting for the glass plate, B, a shallow vessel into which the receiver, C, fits loosely, and is supported on three pieces of cork. This outer vessel serves to receive the overflow water, and any moisture which may condense on

the sides of the vessel which contains the ice. A good microscope, with one-twelfth oil immersion, and preferably Abbé's illuminating apparatus, is necessary. The instrument, made by Zeiss, of Jena, can be thoroughly recommended.

The sterilisation of meat peptone gelatin, &c., is best accomplished by steam at 100° C. in the apparatus Fig. 8, which consists of a metal vessel ½ to 1 metre in height, provided with a tightly-fitting lid, and covered with thick felt. The apparatus is filled about one-fourth with distilled water, and heated by a gas or other flame sufficiently powerful to make the water boil rapidly, and this temperature is to be maintained during the entire period required for sterilisation. A thermometer in the lid indicates the temperature of the steam. Into the interior of the boiler, but above the level of the water, fits a tin vessel (Fig. 9) with a perforated bottom. Into this case the article to be sterilised is placed, and the cover fixed. The case can then be lowered into the boiler by means of the wire handle.

Thin *cover glasses*, and plain and cupped slides, together with glass rods six inches long, with a platinum wire about three inches in length fused in at one end, must also be provided. The wires should be of different thicknesses.

II. *Preparation of Reagents.*

*Meat Peptone Gelatin.**—A ten per cent solution of gelatin in meat juice is perhaps the best strength for water analysis; it is prepared as follows:—Juicy lean meat, carefully freed from fat, is to be chopped up fine, and 250 grms. placed in a beaker with 500 c.c. of cold distilled water. The vessel is then covered, and the mixture allowed to stand for twelve hours in a cold place. In warm weather the mixture should be kept in a refrigerator at a temperature of about 15° C. to prevent decomposition.

Place a clean strong cloth of fine material over a funnel, and pour on it the meat mixture, collecting the filtrate in a graduated cylinder of 500 c.c. capacity. Press the meat until the cylinder contains 400 c.c. If this amount cannot be obtained by manual pressure, the cloth and its contents may be pressed in a small meat-press until the requisite amount of juice has been expressed. As meat-presses are not always available, the pulp in the cloth may be moistened with a small amount of cold distilled water, and re-pressed until the requisite amount of liquid has been obtained.

* Carefully prepared and sterilised meat peptone gelatin can be obtained from Dr. H. Rohrbeck, of Berlin, in test-tubes containing 10 c.c., at 1 mark for 5 tubes, or in flasks at the rate of 2·50 marks for 250 grms.

Pour the 400 c.c. of juice into a wide-mouthed flask, and add:—

* Gelatin	40 grms.
Peptone..	4 ,,
Common salt	2 ,,

Plug the flask with cotton-wool, and allow it to stand on a water-bath at a temperature of 50°—60° C., with occasional agitation, until the gelatin has dissolved, and an uniform mixture results. Direct application of a flame to the bottom of a flask must be avoided, because the gelatin, being in a viscid condition, is liable to burn, and because the temperature of the fluid at this stage of the process must not reach the point at which albumen coagulates.

When the gelatin has completely dissolved, the liquid must be neutralised by carbonate of soda. The carbonate of soda in strong solution is cautiously added with constant agitation, until a drop of the liquid placed upon red litmus-paper gives a *slight blue* reaction. With blue litmus-paper there should be no reddening.

The flask containing the neutralised juice is again plugged and heated to 100° C. in a water-bath, and kept at that temperature for about half an hour. During the heating the albumen is coagulated, and rises to the surface as a dense coagulum. Care should be taken to avoid agitating the liquid, which would cause the coagulum to break up into fine particles, and thereby render the subsequent filtration difficult. If the heat has been applied sufficiently long to coagulate the whole of the albumen the liquid presents the following appearance:—On the surface there is a dense whitish coagulum, while the liquid below is bright, transparent, and yellowish.

The coagulum is to be removed by filtration through fine muslin, and the somewhat turbid filtrate is then to be passed through a moistened ribbed paper filter, surrounded with a hot-water jacket (Fig. 10), the liquid being received into a flask. During filtration the mouth of the funnel should be covered with a glass plate. It is always advisable, before commencing the filtration, to re-test the reaction of the liquid, and if it be found acid to neutralise. It often occurs that the first portions of the liquid which pass through the filter are turbid, in which case the filtrate must be returned to the filter, and this must be repeated until it filters bright.

* Ordinary gelatin does not answer well; that sold in thin transparent sheets, manufactured in France, *premiere qualité*, is best adapted for this purpose.

The flask containing the clear filtrate is plugged and placed on a sand-bath, and the contents boiled rapidly for ten minutes; or the flask may be placed in the steam sterilising apparatus, and heated to 100° C. for half an hour. After this heating, the liquid, while still hot, is to be decanted into the sterilised test-tubes, either directly from the flask or by means of the apparatus, Fig. 11.

In order to fill a test-tube, remove the cotton-plug, taking care not to place it upon the table, but to hold it between the fingers, and to avoid touching that portion which fits into the tube. The tube should then be filled rapidly about one-third with the liquefied gelatin, and immediately re-plugged. After a sufficient number of tubes have been filled, the contents must be sterilised, and this may be done in one of two ways,—by packing the tubes in the tin vessel (Fig. 9), closing the cover, and heating to 100° C. in the steam sterilising apparatus for thirty-five minutes, on three occasions, at intervals of twenty-four hours; or the contents of each tube may be boiled for five minutes over a flame on three occasions at intervals of twenty-four hours. The first-described method is the less troublesome, and is to be recommended.

This method of *fractional sterilisation* has been shown by Pasteur to be surer than the same amount of continuous heating. After sterilisation the tubes should be kept standing on a layer of cotton-wool contained in a beaker or other convenient vessel. The gelatin mixture will remain fit for use for two or three months, but care should be taken to prevent evaporation by slipping over the plug a small cap of caoutchouc.

Sterilised distilled water may be prepared by either direct boiling or in the steam sterilising apparatus. In either case fractional sterilisation must be adopted, the procedure being similar to that employed for the sterilisation of gelatin. Sterilised distilled water is used in certain cases for diluting a sample of water before admixture with gelatin. The sterilised distilled water is best prepared and preserved in test-tubes or small flasks of one or two ounces capacity.

Bouillon is necessary if the development of micro-organisms in capped slides is to be watched. It is prepared from meat-juice, with the addition of 4 per cent peptone, but no salt or gelatin is added: it is neutralised and sterilised in the same manner as the peptone gelatin, but does not require a hot-water jacket during filtration. It is best preserved in small sealed tubes, holding about 20 or 30 c.c. When required for use the contents of a tube may be emptied into a sterilised test-tube, and if the precaution be taken to boil the contents of the tube for a

FIG. 10.

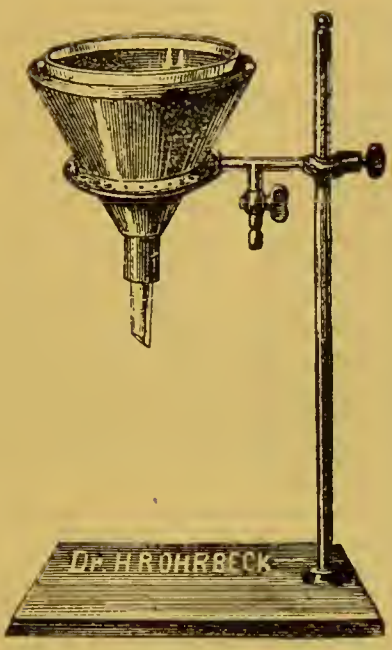
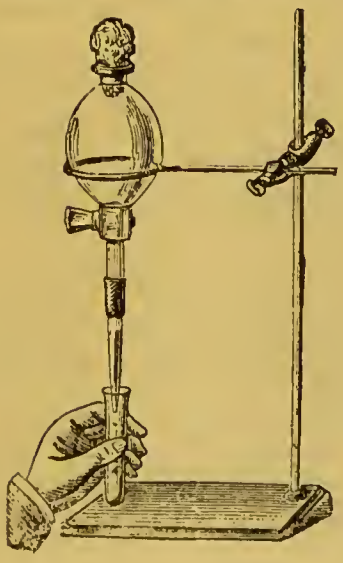


FIG. 11.



few minutes each time the cotton-wool plug is removed to withdraw a drop, the bouillon will keep sound for weeks.

Sterilised potatoes may be required in certain cases for the cultivation of micro-organisms which occur in water, in order to distinguish between varieties which possess similar morphological characters. For instance, several varieties of comma-shaped bacilli are known, among which is the cholera bacillus, but its mode of growth on sterilised potatoes under different physical conditions is one of the points which serve to distinguish it from other similar shaped organisms. Potatoes are prepared for use in the following manner:—They are first well scrubbed with a brush and plenty of water to remove adherent earth, &c.; the "eyes" are cut out, and the potatoes soaked for forty or fifty minutes in a 2 per 1000 aqueous solution of sublimate; washed again, placed in the tin vessel (Fig. 9), and heated in the steam sterilising apparatus at 100° C. for one hour. The vessel is then removed from the steam-bath, and the contents allowed to cool with the cover on.

Aniline colours are employed for staining the bacilli. Methylen blue, gentian-violet, or fuchsine may be used, but the first-mentioned dye is the most useful. For permanent mounted cover glass preparations, however, methylen-blue has the disadvantage of fading more rapidly than the other colours. About 2 grms. of the dye should be rubbed up in a mortar with 10 c.c. of absolute alcohol, 90 c.c. of distilled water gradually added, and the mixture filtered through paper into a bottle provided with a perforated cork carrying a pipette, by means of which a small amount of the dye solution can be removed as required. Or saturated alcoholic stock solutions of the dyes can be prepared and diluted in the proportion of 1 to 9 with distilled water. A small fragment of camphor should be placed in the filtered solution; it prevents the formation of mould, and also appears to favourably influence the colouration of the bacilli.

III. *Collection of Samples.*

I would strongly recommend that the collection of samples should always be personally conducted, as special precautions must be taken to avoid accidental contamination of the water with foreign germs. Unless the operator be certain that the sample has not been contaminated the results of the analytical process are worthless. Micro-organisms are naturally present in air, water, and on every surface which has been exposed to the atmosphere, and it is quite conceivable that a person unacquainted with methods of bacteriological research might

use distilled water to clean a flask intended for the reception of a sample for biological examination, under the impression that, because distilled water is nearly chemically pure, it must also be germ free. But as a matter of fact ordinary distilled water, as found in laboratories, teems with micro-organisms.

The sources from which water may have to be drawn for bacteriological examination may be arranged under the following heads :—

A. Hydrant or tap water.
B. Water of rivers and lakes.
C. Well waters.

It may be laid down as a general rule in drawing a sample, that, whatever be the source from which the sample is taken, contact of the water with vessels other than the sterilised flasks in which it is to be preserved should be avoided.

In the Gesundheits Amt, Berlin, Erlenmeyer's flasks (Fig. 12), of about 4 ounces capacity, are used for the collection of samples. The flasks are cleaned, plugged,

Fig. 12.

and sterilised in the same manner as the test-tubes used for holding the meat peptone gelatin. When cold, over the mouth and cotton-plug of each flask a tightly-fitting cap of black caoutchouc is fixed, and in this state the flasks are issued.

When it is necessary to take a sample of water from a tap, or " stand-pipe," the water should be allowed to flow in a full stream for about ten minutes. The rate of flow is then diminished, and the cap of caoutchouc and plug removed from the mouth of the flask, and the water allowed to flow directly into it until it is about two-thirds full, and the plug and cap immediately fixed. The mouth of the flask should not be allowed to come into contact with the tap.

In collecting a sample of river or lake water the neck of the flask is preferably fixed in a clean split stick ; the

flask is then inverted, and the cap and plug removed, and the flask forced below the surface of the water; the position is then reversed, and the flask removed filled with water. A small portion of the contents is then poured off, and the flask immediately re-plugged and capped.

In collecting a sample of water from sources included under the heading B, care should be taken to avoid the proximity of the banks, outlets of drains, &c.—unless there be special reasons. Probably a sample taken from the middle would represent better the normal condition of the water. But, obviously, should the water be drawn for domestic purposes from a landing-place, or from the banks, then a sample should be taken from that locality, and a second from mid-stream, and both examined. When a sample is drawn from a boat it should be taken from the bows.

In drawing a sample from a well the flask should have a loop of twine fixed round the neck, and a second one lower down around the body; the string by which the flask is lowered being attached to the upper loop, and a short length with attached weight, as a sinker, to the lower loop, but tied on the opposite side to the twine by which the flask is lowered.

The method of collecting samples in Erlenmeyer's flasks is only applicable when the transport of the flask without inversion can be assured: when samples have to be forwarded long distances the method must be modified. As the amount of water necessary for a biological examination is small, bulbs of glass, of about 5 or 6 c.c. capacity, can be used for collection and transport. A bulb of approximately the requisite size is blown at one end of a piece of soft glass tubing, 6 inches in length. The tube is now held by a pair of forceps, and the bulb and whole of the tube strongly heated; the open orifice is then fused, and drawn out into a capillary tube of about 2 inches in length, and the end sealed by fusing the glass. Tubes of this form suitably packed in cotton-wool may be sent long distances by post with safety. They are filled in the following manner:—Hold the tube a short distance above the bulb, and pass the capillary and upper part of the tube repeatedly through a flame. Allow the end to become quite cold, care being taken during the cooling to avoid contamination. Then plunge the capillary end about 2 inches below the surface of the sample, and with a pair of previously sterilised forceps nip off the end below the level of the water. As the tube was sealed while hot, and therefore partly exhausted of air, when the end is opened below the surface of water a certain quantity enters. The tube is now removed and the bulb held downwards, and, with one or two jerks, any

drops remaining in the capillary end shaken down; the capillary orifice is then closed by fusing the glass, and the tube thus hermetically sealed.

Another method of preparing bulbed tubes for the collection of samples is as follows:—After a bulb has been blown a small amount of sterilised distilled water is introduced, and the open end drawn out into a capillary tube. The water in the bulb is then boiled, and after a short time the bulb removed from the source of heat, and the capillary end sealed at the moment ebullition ceases. By this plan a better vacuum is obtained.

IV. *Analytical Process.*

In Prof. Koch's earlier method for the bacteriological examination of water, the specimen was mixed with the liquefied nutrient gelatin contained in a test-tube. On the gelatin solidifying each micro-organism was fixed, and developed into a colony, and from the appearance presented by the tube after the lapse of a few days a rough idea was formed of the number of germs present. But this method, though perhaps occasionally useful, lacks precision: the number of colonies of micro-organisms cannot be estimated, and the description of the appearance of the tube must be left to the judgment of the operator; the results, therefore, have no quantitative value, and are not comparable. By the employment of glass plates, on the other hand, to receive the liquefied gelatin after admixture with the water, the development of the bacterial colonies may be watched, the morphological characters of the organisms ascertained, other culture media may be inoculated from individual colonies, and lastly, the number of germs present in a given volume of water may be estimated; the results, therefore, arrived at by different operators are strictly comparable.

The first step before admixture of a sample of water with the nutrient gelatin is to ascertain approximately the number of micro-organisms present, for on the number depends the volume of water which will have to be employed for the analytical process. If the sample be contained in an Erlenmeyer's flask, the caoutchouc cap is removed, the cotton-plug singed, and the mouth of the flask heated by a Bunsen or other flame. The plug is then withdrawn with a twisting movement, the flask being held slanting: with a sterilised glass rod or pipette a drop of water is removed, placed on a clean cover-glass, and allowed to dry under a bell-jar. When the water has evaporated, one corner of the cover-glass is held by a pair of forceps, the side containing the residuum being upwards, and the glass rapidly drawn *three times* with a

downward motion through a colourless Bunsen, or large spirit-flame. The cover-glass, still held by the forceps, is then flooded with methyl-blue solution, which is allowed to act for about three minutes. The dye is then washed off by a gentle stream of water, and the cover-glass mounted on a slide with a drop of water, and examined with a 1/12 oil immersion system. If it be thought desirable to preserve the specimen, the cover-glass should be taken off from the slide, oil removed from the back, and the glass allowed to dry by exposure to air, and ultimately mounted in Canada balsam dissolved in xylol. Care must be taken not to employ heat during mounting, else the bacilli will be decolourised.

On microscopic examination of a cover-glass preparation, if one bacillus be detected in each field, one drop of the water will contain many hundred, and less than one cubic centimetre will have to be taken for mixing with the gelatin. On the other hand, should several "fields" have been examined without detecting a bacillus, then 1 c.c. of the water should be taken. It is difficult, however, to lay down specific rules regarding the exact volume of water to be employed. It is better to take too little than too much, because in the latter case the colonies which develop in the gelatin are so close together that they coalesce, and the examination then becomes difficult or impracticable.

A drop of the water should also be examined in a cupped slide in order to ascertain the motility of the organisms. For this purpose the edge round the depression in a cupped slide is painted with vaseline, and a drop of water placed in the centre of a cover glass, which is then inverted, and fixed over the hollow by the layer of vaseline. The preparation is then examined with a 1/12 oil system, and a diaphragm with a very small aperture before the substage illuminating apparatus. In the periphery of the drop the movement of the organisms will be best visible.

Examination of the stained cover-glass preparation and of a drop of the water mounted in a cupped slide will lead to the detection of—

Bacilli movable and stationary, of different size, shape, and thickness, single, in groups, or chains.
Micrococci, single, in chains, or zooglæa form.
Vibrios.
Spirilla.
Algæ, &c.
Amorphous and granular detritus.
Crystalline bodies.

Care must be taken not to confound the Brownian movement of inorganic particles for the motion of micrococci; and the distinction of bacteria from inorganic substances is also a point which needs attention. Dr. Sternberg* remarks:—"The smallest forms of bacteria may he confounded with various matters, with organic particles, molecular granules, fat globules, &c. These productions, which are found in considerable quantity in the liquids or in the tissues of animal or vegetable origin, often resemble so closely in form, size, and grouping the spherical bacteria, that it is absolutely impossible to guard one's-self against confusion unless the most minute precautions are taken in making the observations (Cohn). The detritus, the amorphous powder, of precipitated molecules of inorganic substances, even when they exhibit the Brownian movement, are easily enough distinguished from micrococci by optical signs, their angular form, their less refractive power, and finally by their reaction with certain chemical agents; above all, if they are mineral substances, crystalline bodies, &c. It will not be the same with molecular granules of organic nature. They have a common character, their rounded form, their notable refractive power, movements. Nevertheless, their form is less regular, more angular, their colour variable, their refractive power always less. In doubtful cases, Tiegel has given a method which enables us to distinguish them from micrococci. It consists in warming the glass slide which supports the corpuscles under examination; if they are 'coccos' they are seen to move in a manifest manner. This does not occur in the case of molecular granules.

"Hiller, who has paid especial attention to the means of recognising bacteria, divides them into two groups:—

"A. The optical signs: comprising—(1) The characteristic vegetable form, rods, leptothrix; this he recognises as of little use, as in this case there is no doubt. (2) The characteristic movements of the monads. (3) The mode of growth and of multiplication. (4) The mode of junction of the granules.

" The chemical signs:—(1) False zooglæa become softened and diffluent under the action of liq. potassæ, and are coagulated by direct application of alcohol. (2) In sections of tissues after an hour of maceration in liq. potassæ, diluted one-tenth, the monads are coloured brown by iodine, while fat granules are not.

* Bacteria," by Sternberg and Magnin.

"But, in truth, the method of cultivation extolled by Cohn and Wolff is the best means of distinguishing bacteria. The distinction of pseudo-bacteria," says the first of these authors, "from veritable globular bacteria is a problem which our microscopists cannot re-solve, in any case with desirable certainty. It is only by a study of their mode of development that this distinction can be made. *The globules which divide and develop in form of chains are organised beings; when this does not occur, we are dealing with pseudo-bacteria.* This, however, is not exactly the opinion of Nägeli, who seems to consider movement as the surest distinctive characteristic. 'There are,' he says, 'but three distinctive signs which enable us to recognise with some certainty that granules under observation are organisms, —spontaneous movement, multiplication, and equality of dimensions, united with regularity of form.

"The most certain sign is movement in a straight or curved line—a movement which inorganic granules never present. One should take care not to be deceived by movements which are caused by currents in the liquid under observation. Nor should one allow himself to be deceived by the tremulous motion, called molecular movement, in which the granules do not really change their position. These movements are seen in most cells, and even in those of the Schizomycetes, and inorganic bodies themselves present it.

"Multiplication is a character less important than movement. When among granules some are found united in pairs, it may be supposed with probability that division and multiplication are taking place. When rods are bent at an angle, one may predict their division into two parts.

"Finally, as to size and form. Granules of different size and of a more or less irregular form ought not to be considered as belonging to the group of segmented fungi; if, on the contrary, the granules offer dimensions perfectly equal, and a spherical or oval form, the distinction is more uncertain: they may belong to the schizomycetes or be of inorganic nature."

If 1 or 0·5 of a c.c. of the water be employed for the analytical process, the volume can be measured from a divided pipette. If smaller volumes be used, it is better to dilute the sample with sterilised distilled water, so that 1 c.c. of the mixture shall represent the requisite volume of water, than attempt to deliver from a graduated pipette one- or two-tenths of a c.c.

The volume of water to be used is now to be mixed with the gelatin. For this purpose the contents of a test-tube containing meat peptone gelatin is liquefied either by cautious direct application of a flame, first to the upper

and then to the lower layer of gelatin, care being taken to avoid boiling, which would cause the formation of bubbles; or, preferably, by immersing the lower part of a tube in water heated to 30° C. After the tube has stood for some minutes, ascertain, by inclining it, whether the whole of the gelatin has been liquefied or not. This precaution should always be taken, more especially, perhaps, when direct application of a flame has been used to liquefy the gelatin, otherwise a plug of solid gelatin may remain in the centre. If the whole of the gelatin has been liquefied, wipe the tube, twist the cotton-plug round without withdrawing it,—to break down any adhesions it may have contracted to the glass, by the sides having been accidentally moistened with gelatin,—and pass the upper part of the tube through a flame two or three times so as to singe the plug, and so destroy any germs which may have been deposited upon it or on the lip of the tube. Allow the tube to stand for a few minutes to cool, then, holding it in the left hand in a slanting direction, remove the plug with a twisting movement, and place it between the first and second fingers of the same hand, and allow the water from the sterilised pipette to flow down the side; immediately replace the plug, and move the tube to and fro several times in order to thoroughly mix the contents.

The gelatin water mixture is then poured on the centre of a sterilised glass plate supported on the levelling apparatus (Fig. 7), and with a sterilised glass rod evenly spread so as to form a square of about 9×9 c.m., thus leaving an uncoated margin round the plate This operation must be rapidly performed, as the gelatin, owing to the low temperature, sets quickly. Care must be taken to prevent the formation of air-bubbles. The coated plate is covered with a bell-jar (E, Fig. 7), and after the lapse of about four minutes placed upon a glass bench (Fig. 4), on which has been previously laid a slip of filter-paper, giving particulars of the nature of the experiment, and the plate supported on the bench introduced into a moist chamber, which is immediately closed. A second plate and bench may then be placed over the first, and so on until the chamber is filled.

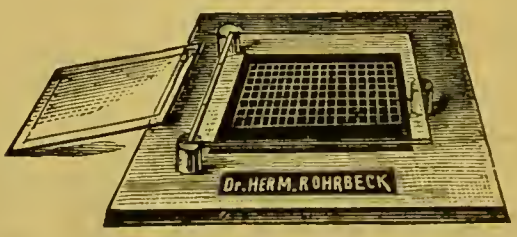
The volume of liquefied gelatin which is spread over a plate of the dimensions prescribed should not be less than 10 or 11 c.c.; if the layer be too thin it dries rapidly, and the growth of the micro-organisms is retarded; an unduly thick stratum also acts prejudicially.

The moist chamber should be kept in a moderately cool room; a temperature of 60° to 65° F. is the most suitable. A very low temperature retards growth, or may even prevent the development of colonies. After the lapse of 24

to 30 hours, depending upon the temperature, the plates should be examined. The colonies at first appear as minute whitish spots, which gradually increase in size. Certain colonies will liquefy the gelatin, and the liquefied portions will be either colourless or present a greenish yellow fluorescence; or more rarely a violet colour may be produced. Others will remain solid. After about 72 hours, when the colonies have reached a sufficient size so as to be visible without difficulty to the naked eye, their enumeration should be made.

If the colonies be few in number they can be at once counted, but when a large number have developed the contrivance shown in Fig. 13 is useful. The apparatus consists of a wooden frame, on which is fixed a glass plate ruled by horizontal and vertical lines into squares of 1 centimetre, some of the squares being again subdivided. Behind the plate is placed a piece of dull black cloth or paper. The gelatin plate on which the colonies are to be

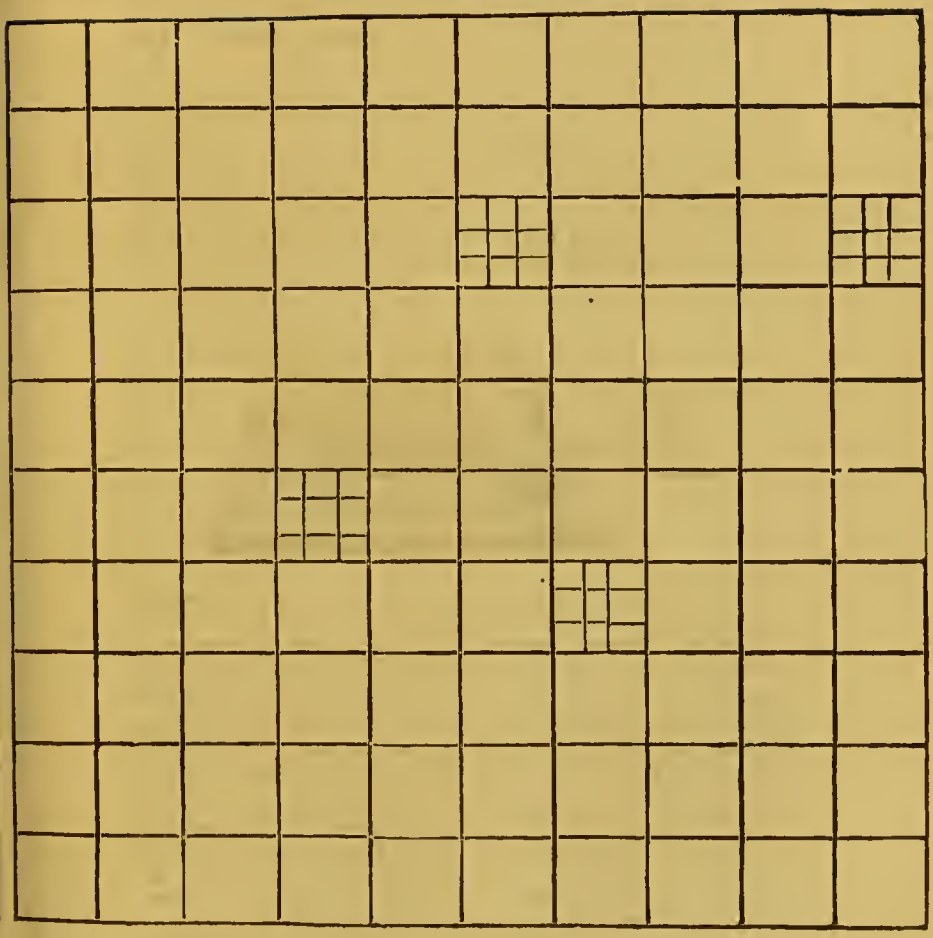
Fig. 13.



counted is placed on the ruled plate, and the number of colonies which fall in one line of vertical and one line of horizontal squares counted, and the figures so obtained multiplied, the result representing approximately the number of germs originally present in the volume of water employed for the analytical process. Unless the colonies are very evenly distributed over the surface of the plate it is better to take the mean number of colonies found in two or three lines of horizontal and the same number of vertical line squares, and to multiply the means together. It frequently happens that a colony falls on a line, in which case some such rule as the following should be adopted :—Colonies falling on a line to the left, in a vertical square, are to be counted, those to the right excluded. A similar rule is also applicable to the lines bounding the horizontal squares.

An efficient substitute for the apparatus described as Fig. 13 is made by ruling on a sheet of dull black paper pasted on cardboard, a square 10×10 c.m. divided into

Fig. 14. (Original Size.)



100 squares of 1 cubic metre each, a few of the squares being subdivided as is shown in Fig. 14.

After the total number of colonies has been estimated, the enumeration of those which have liquefied the gelatin should be made. The results may be recorded in the following manner:—

One cubic centimetre of the water contained ——
Colonies of which ——
Liquefied the gelatin ——

A record should also be kept of any peculiar appearance, colour, or odour, &c., of the colonies.

Although mere enumeration of the colonies developed on a plate affords valuable indications, yet the maximum amount of information which the method can yield is by no means exhausted. Bacteria are normal constituents of natural waters; and the information which is of importance is not only the number present above the normal, but also the kind. A water teeming with micro-organisms might be drunk with impunity; but if a water contained micro-organisms much below the normal in number, but which could be identified as being similar to the organisms found —for instance, in the cholera process the use of such water would certainly be attended with danger; for leaving aside the question whether the cholera bacillus is or is not the cause of the disease, the fact remains that the organism described as the Cholera Bacillus by Dr. Koch occurs only in Asiatic cholera, and its detection consequently in water is *primâ facie* evidence of contamination with Cholera Dejecta. It is therefore of moment that the examination of a water should not be considered as completed with the mere enumeration of the bacteria.

After the first appearance of the development of colonies a plate should be microscopically examined at regular intervals, and the general outline, refractive power, and mode of growth of the colonies watched. For this purpose the plate is placed on the stage, and examined with a low power,—Zeiss's A A system, with No. 3 eye-piece magnifying 71 diameters, being sufficient, a small diaphragm being fixed below the sub-stage illuminating apparatus. The morphological characters of the organims which form the colonies should also be examined; and this may be accomplished in one of two ways:—A cover glass may be pressed over a colony, removed with a pair of forceps, passed three times through a flame, then stained in the manner already described, and examined, mounted in a drop of water, with a 1/12 oil immersion system. This method, while permitting the morphological characters of the organism to be viewed, also shows its disposi-

tion in the colony. Or, while the plate is on the microscope stage, and a particular colony in the field, a portion of the colony is removed by a platinum wire, and rubbed over a cover-glass, which is then heated and stained in the usual way. This procedure is especially useful when the colonies are close together, and it is desirable to examine the contents of one only. The platinum wire should be moderately thick, and mounted in a glass rod, the wire at about one line from the free end being bent nearly at right angles. The wire is heated red-hot, allowed to cool, and the glass rod held between the thumb and fore finger of the right hand, the little finger resting on the side of the stage. The bent point of the wire is now brought into contact with the centre of the object-lens, the eye applied to the eye-piece, and the point of the wire carefully depressed until it appears over the colony, when it is still further depressed so as to be engaged in the colony. The eye is then closed, the point of the wire raised, and the trace of the colony removed, rubbed over a cover-glass, which is then heated and stained. The colony which has been operated upon is then re-examined, to ascertain whether the operation has been properly performed or not. If a large amount of gelatin be removed with a colony there is some difficulty in fixing the organism on the cover-glass. After staining, and on washing the cover-glass, nearly the whole of the stained colony will be washed away. This is best obviated by placing a small drop of bouillon on the cover-glass, and then mixing the portion of removed colony with it, and spreading the mixture over the glass. After drying, the cover-glass is heated and stained as usual. Or, after staining, the cover-glass, with the dye solution still on it, is inverted on a slide, and the dye liquid soaked up with blotting-paper: a few drops of water are then placed near one of the edges of the cover-glass, and removed from the opposite edge by blotting-paper, and this is repeated until the superfluous dye solution has been removed.

The mode of development in bouillon of an organism removed from a colony may also be necessary as a means of diagnosis. The bouillon is inoculated within the organism by touching a colony with a sterilised platinum wire, and then bringing it into contact with a drop of bouillon on a cover-glass, which is then inverted over a cupped slide in the manner already described. The cupped slide may be kept at a temperature of from 25° to 30° C., and examined at intervals of about twelve hours with a 1/12 oil immersion system, and a very small diaphragm below the sub-stage illuminating apparatus. The motility, formation of spores, development of spirilla, and mode

of division of the micro-organism will by this means be ascertained.

The mode of growth of an organism on a sterilised potato also affords useful information. Certain organisms grow well at ordinary temperatures, others only develop at a high temperature. Some form a coloured layer on the surface of the potato; in others, at a high temperature, a large development of spores occur. In order to inoculate a potato, a sterilised potato, prepared in the manner already described, is held between the fingers of the left hand,—previously washed with corrosive sublimate solution,—and divided by a sterilised knife. A rather large amount of the colony is then placed upon the centre of the potato, and with a sterilised scalpel spread over the surface, leaving a free margin round the potato. The inoculated potato is then placed in a moist chamber, and kept at either an ordinary or a high temperature, as the case may be. Fractional cultivation on a potato is performed by transferring a small fragment of the mixture of potato pulp and colony from the surface of the first potato, and placing it upon a second, over which it is spread; from the second potato a portion is then removed to a third. For each potato divided, and for each portion of inoculated potato pulp transferred, a freshly sterilised knife and scalpel must be employed.

The mode of growth of an organism in a test-tube in gelatin is also of importance. A test-tube containing sterilised gelatin is inoculated in the following manner:—The test-tube containing solid gelatin is inverted, the cotton plug removed, and the substance with which the gelatin is to be inoculated touched with a sterilised platinum wire mounted in a handle, which is then pushed through the gelatin, removed, and the tube re-plugged.

In the ordinary course of an analysis it is by no means rare to find on the surface of the gelatin plate a few growths of micro-organisms which have been derived from the air, and which may consist of moulds, or colonies of bacteria, and sarcina, &c. It will be observed that all these growths have sprung from the *surface*, and not from the *interior* of the gelatin. It is necessary, however, that the analyst should be familiar with these organisms which occur in air, and for this purpose—before commencing to examine a sample of water—to make a few blank experiments in the following manner:—Prepare one or more plates without admixture of water with the gelatin, and keep the plates in a moist chamber. In a second experiment expose one or more plates coated with gelatin to the air, for varying periods. If no development occurs on the plates of the first experiment it is evidence that

the reagent and apparatus have been properly sterilised. The growths on the plates in the second series will have been derived from the air.

It may be of interest to append the appearances presented by the cholera and typhoid bacillus when cultivated in different media. Dr. Koch found the cholera bacillus in the water of certain tanks in India: he also found in one sample a bacillus, which, while agreeing in morphological characters with the cholera bacillus, proved on cultivation not to be that organism. The necessity, therefore, of not assuming on morphological characters alone that every comma-shaped bacillus is the cholera bacillus must be kept in view.

Cholera Bacillus; Growth on Plates.—At a very early stage the colonies appear as very pale and tiny drops, which are not quite circular, and present a granulated appearance. As a colony grows the granulated appearance becomes more evident, and it looks like a mass of refracting granules. At a later stage the gelatin liquefies in the immediate neighbourhood of the colony, and it sinks into the gelatin. A funnel-shaped cavity is thus formed, and at the apex a small whitish spot is seen. The gelatin liquefies very slowly. During the growth of the colonies a peculiar odour is slowly developed, which is not unlike the smell of a fresh cholera intestine.

Growth in Test-tubes.—As soon as the cultivation commences to develop a small funnel-shaped cavity is formed, which marks the site of inoculation. By degrees the gelatin liquefies in the neighbourhood of this point of inoculation, and the colony extends along the track of the inoculating needle. But there is always a deep spot, sunken at the top, and the border of the funnel-shaped cavity is always formed by a ring of solid gelatin. Three strata may be distinguished in this funnel-shaped cavity: an upper turbid layer, containing active bacilli; a middle translucent or nearly clear layer; while the apex of the funnel is occupied by a loosely-packed white *débris*. At temperatures from 17° to 19° C. growth is very slow.

Growth on Potatoes.—There is little or no growth at a temperature from 17° to 19° C. At a temperature, however, of 30° C., after some days, small brownish colonies appear, but always in thin layers.

Growth in Cupped-slides with Bouillon.—The cholera bacillus exhibits rapid motion, and at a high temperature there is a large development of spirilla, which are not simple wavy threads, but very tender long spirals, which move in an animated manner.

Morphological Characters.—The cholera bacillus is about half, or at most two-thirds, as long as a tubercle bacillus, but much more bulky, thicker, and slightly curved. Curve generally not more marked than that of a comma, but may be semi-circular, or curve may be double, like a letter S.

As regards the detection of the typhoid bacillus in water, Dr. Gaffky informs me that the examination of several specimens supposed to have been contaminated with typhoid dejecta, led in no single instance to a positive result. This is, however, no reason why the search should be abandoned. Scientifically it would be of the highest interest to be able to demonstrate the presence of the typhoid microbe in a medium which is so frequently credited with conveying the disease.

Typhoid Bacillus: Growth on Plates.—Small yellowish brown colonies in the interior of the gelatin, which attain their maximum growth in from four to eight days, and then do not increase in size, and which do not cause liquefaction of the gelatin. On examination the colonies appear somewhat granular, roundish, and as if filled with densely interlaced fibres, which come to the surface, grow on it, but do not extend any distance round the borders of the colony.

Growth in Test-tubes.—The bacilli grow along the track of inoculation, but not very luxuriantly; appear on, and form a thin layer over, the whole surface of the gelatin. Do not liquefy the gelatin.

Growth on Potatoes.—After two or three days at ordinary temperatures, no appearance of growth to naked eye; on microscopic examination, however, the whole surface of the potato is found covered with more or less actively mobile bacilli. Below 20° C. no spores are found, but at 30°—42° C. spores develop after three or four days. The spores are round at the ends, and occupy the entire diameter of a bacillus. Long filaments of jointed bacilli are often developed, in which case the spores are at opposite ends; while at the point of contact of two bacilli, commencing spore formation is visible.

Growth in Cupped-slides.—If inoculated in bouillon from potatoes, the bacilli appear thicker than when seen in the spleen or other organs. Actively mobile, best seen at periphery of the drop, to which the bacilli appear to lie parallel; of different lengths; jointed threads also formed. No spore formation at low temperatures.

Morphological Characters.—In length about one-third the diameter of a red blood cell, and three times as long as broad; ends distinctly rounded. As a rule do not stain well, and very frequently not uniformly, unstained

circular spots not extending the whole diameter of a bacillus being left pale. Methyl-violet is perhaps the best stain.

V. *Inferences to be drawn from Results.*

It has already been pointed out that micro-organisms are normal constituents of water, and in drawing conclusions from the results of analyses, both the number and the character of the bacilli must be taken into consideration. As regards number, there is evidence to show that the purer the water the smaller the number of bacilli present. The variations in number are shown in Table No. I.* As regards general characters of the bacilli, at present it is assumed that those which liquefy the gelatin are more harmful than those which do not. But it appears to me that the evidence on this point is not conclusive. An alteration in the chemical composition of the culture-gelatin is apparently sufficient to cause colonies which did not liquefy ordinary meat peptone gelatin, to liquefy the modified culture medium. In Table No. II.† the results of the chemical and biological examination of fourteen samples of water from various sources are tabulated.

The results of analyses 13 and 14 in Table No. II. are interesting, and demonstrate in a striking manner the effects of efficient filtration in removing micro-organisms.

The employment of the biological method for the examination of water supplied by public companies, ought on these grounds to prove useful. As a means, also, of ascertaining the relative value of domestic filters, the bacteriological examination of the water before and after filtration appears to be the only method likely to afford reliable indications.

In conclusion I would gratefully acknowledge the very great obligations I am under to Prof. Koch and to Dr. Gaffky for the valuable instruction I received at their hands during the period of my study at the Gesundheits Amt Berlin.

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* From the "Bericht der Deputation für die Vermaltung de Kanalisationswerke für der Zeit vorm 1st April, 1882, bis zum 31st May, 1883.

† *Ib.*

TABLE I.

Source of the water.	Number of germs in one cubic centimetre.
Water from the pressure tube *(druckrohr)* in Falkenberg—	
In August	50,000,000
In October	46,000,000
The ditch in which drain waters of the filtering fields near Falkenberg are collected	44,000
Between Falkenberg Marzahn	76,000
The water from separate drain pipes at Falkenberg	460
	860
	4400
	48,000
	420,000
The water of the Wuhle..	830,000
The Spree above the entrance of the Wuhle	210,000
The limiting ditch	616,000
The Spree in the town—	
Above Panke	940,000
Below Panke	1,800,000
The Spree at Belleme—	
First examination	1,640,000
Second examination	4,480,000
The Spree at Charlottenburg	10,180,000
The Spree at Spandau—	
First examination	220,000
Second examination	5,000,000
The hydrant water in the Gesundheits Amt Berlin from Tegeler lake water in many examinations..	160 to 250
Tegeler lake	3740
Well water from different wells amounted to	40 to 160
But reached in some cases	4000 and 12000

TABLE II.

Source of the water.	Colour.	Odour.	Transparency.	Hardness. Total.	Hardness. Perman't.	Per 100,000 parts. Total solids at 100° C.	Per 100,000 parts. Cl.	Per 100,000 parts. NaCl.	Parts. SO_3.	Parts. N_2O_5.	Parts. N_2O_3.	Parts. NH_3.	Reducing power of organic matter present in 100,000 parts of the water on $KMnO_4$.	Microscopic examination of the fresh water in a cupped slide, and also of dry stained cover-glass preparations.	Number of germs present in *one* cubic centimetre of the water.
1. Liquid sewerage from the pressure tube — before filtration.	—	Putrid odour on long standing, which increases.	Dark flocky masses.	—	—	256·46	—	—	—	—	—	—	—	Numerous movable and stationary micro-organisms, consisting of monads, colourless algæ, bacilli, micrococci in chains, and zooglæa masses. Vibrios, spirilla. Abundant amorphous debris. Around the edge of the dried drop on the cover-glass there was no appearance of crystallisation.	38,000,000 colonies, of which 989,000 liquefied the gelatin.
After filtration through paper.	—	Distinct putrid odour.	Turbid by white flocks, which settle on standing.	13·25	7·28	107·06	23·43	38·625	7·92	0·25	Nil.	8·440	22·12		
2. Water from the N. part of the sewers.	—	Faint unpleasant smell, which disappears on longer standing.	On examination turbid. The turbidity subsides on longer standing and forms a precipitate contng. oxide of iron.	14·16	3·60	83·88	18·46	30·45	5·48	2·12	0·1500	1·60	4·46	Few colourless algæ, separate movable bacilli, few stationary bacilli, few micrococci. Crystalline bodies abundant.	87,000 colonies, of which 3800 liquefied the gelatin.
3. Ditch 20 paces above its entering in the Wuhle.	—	As No. 2.	As No. 2.	14·35	3·90	88·26	18·46	30·45	4·48	1·30	0·1875	1·8	4·28	Colourless algæ, a tolerable number of movable short and thin bacilli, a few larger, partly movable. Pretty large number of micrococci in masses, with detritus. Crystalline bodies in abundance.	409,000 colonies, of which 6500 liquefied the gelatin.
4. Wuhle at the crossing of the road.	—	On first examination, and after keeping, hardly any odour.	On examination slightly turbid; after 3—4 days flocks subside; contain oxide of iron.	15·20	4·0	73·42	14·91	24·57	4·10	0·47	Nil.	1·44	5·69	Single colourless algæ and vibrios, many short fine movable and stationary bacilli. Heaps of micrococci. Crystalline bodies.	55,000 colonies, of which 1650 liquefied the gelatin.
5. Limiting ditch at exit from the filtering fields.	—	On examination slight odour, which is lost after some time.	On examination rather strongly turbid; turbidity subsides after 3—4 days; flocks contain oxide of iron.	15·20	4·0	71·58	14·20	23·40	3·20	0·33	0·0665	1·420	3·99	Single spirilla and colourless algæ; pretty large number of bacilli and micrococci, the first partly movable. Crystalline bodies.	210,000 colonies, of which 3680 liquefied the gelatin.
6. Limiting ditch at entrance into the lake of Rummelsburg.	—	No odour, even after standing.	On examination faintly turbid; after 3—4 days separation of a slight precipitate, which contains oxide of iron.	11·78	3·51	44·18	6·39	10·53	3·20	0·08	Nil.	0·560	2·68	Pretty large number of colourless algæ. Short fine and stationary bacilli. Single groups of large, thick, stationary bacilli. No crystalline bodies.	80,000 colonies, of which 540 liquefied the gelatin.
7. Rummelsburg lake 40 paces below the entrance of the limiting ditch.	Nearly colourless, very faintly yellowish.	No odour, even after standing.	On examination nearly transparent; after 3—4 days very slight precipitate, which contained oxide of iron.	5·33	—	16·60	2·13	3·51	Trace.	Nil.	Nil.	0·028	2·72	Small movable bacilli; very few large micrococci joined in couples. No crystalline bodies.	32,000 colonies, of which 850 liquefied the gelatin.
8. Rummelsburg lake near the ice-works.	As No. 7.	As No. 7.	As No. 7.	5·33	—	19·18	2·13	3·51	Trace.	Nil.	Nil.	0·01	3·44	Same as No. 7.	43,000 colonies, of which 260 liquefied the gelatin.
9. Spree above Köpenick.	As No. 8.	As No. 8.	As No. 8.	5·36	—	19·14	2·13	3·51	Trace.	Nil.	Nil.	0·011	2·77	Pretty many short stationary bacilli. No micrococci. No crystalline bodies.	82,000 colonies, of which 140 liquefied the gelatin.
10. Spree 200 paces above entrance of the Wuhle.	As No. 9.	As No. 9.	Transparent. On standing separation of very few white flocks.	5·36	—	14·34	2·13	3·51	Trace.	Nil.	Nil.	0·008	2·59	Same as No. 9.	115,000 colonies, of which 120 liquefied the gelatin.
11. Wuhle 200 paces above its entrance into the Spree.	As No. 10.	As No. 10.	Nearly transparent. After long standing separation of a few brown flocks which contain oxide of iron.	9·0	2·60	53·10	9·33	15·37	3·0	Nil.	Nil.	1·04	2·59	Pretty many movable and stationary bacilli of middle size; fragments of colourless algæ. Single heaps of micrococci. A slight separation of crystalline bodies.	52,000 colonies, of which 920 liquefied the gelatin.
12. Spree 200 paces below the entrance of the Wuhle.	As No. 11.	As No. 11.	Nearly transparent. After long standing separation of very few yellow flocks.	5·36	—	14·58	2·13	3·51	Trace.	Nil.	Nil.	0·018	2·62	Same as No. 9.	118,000 colonies, of which 95 liquefied the gelatin.
13. Waterworks of Stralau before filtration.	As No. 12.	As No. 12.	As No. 12.	5·36	—	17·0	2·13	3·51	Trace.	Nil.	Nil.	0·011	2·68	Same as No. 9.	125,000 colonies, of which 135 liquefied the gelatin.
14. Ditto, after filtration.	As No. 13.	As No. 13.	Transparent, even after long standing.	5·36	—	16·40	1·99	3·276	0·96	Nil.	Nil.	0·004	2·50	No micro-organisms detected.	120 colonies, of which liquefied the gelatin.